thickness. No signs of lungworm infestation was observed. In addition, feeding FR with FGSM provide more profit of PhP485.00 than those fed FR without FGSM.

Based on the result of both feeding trials, FGSM could replace 10% commercial mash; and 37.5% and 60% soybean oil meal in formulated grower and finisher ration respectively. Thus, FGSM is a good protein source for swine and provide additional income to swine raisers.

Keywords: Golden snail, swine/hog/pig

BIOLOGICAL SCIENCES

BSD No. 1

PARTICIPATORY INVENTORY AND CONSERVATION STUDIES OF ENDEMIC, ENDANGERED AND ECONOMICALLY IMPORTANT FLORA IN SELECTED FORESTS OF MINDANAO

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Mindanao forests have not been spared from wanton destruction due to human activities. An inventory of flora in selected forests of Mindanao was conducted to assess its status and conserve the endangered, endemic, and economically important species in the said mountains.

Through survey, collection and participatory establishment of a 1-ha plot, an assessment of plant diversity in Mt. Musuan, Mt. Kalatungan and Mt. Malindang in Mindanao revealed the presence of 963 species, 231 genera and 182 families. Noteworthy was the discovery of new records of mosses in the Philippines, viz., *Chaetomitrium horridulum*, *Metadistichophyllum rhizophorum* and *Camptochaeta subporotricholdes*. Assessment of conservation status of each species showed 12 endangered species, 252 endemic species, 17 rare species, 187 economically important species and 10 species of socio-cultural importance. An initiative to conserve these endangered, endemic, rare and economically

important species was conducted by propagating them in the garden and in the greenhouse as part of the *ex situ* conservation and as sources of explants for *in vitro* culture. Those plants that were successfully cultured/propagated through *in vitro* were *Lycopodium clavatum* (rare and ornamental fern ally), *Lycopodium cernuum* (medicinal and ornamental fern ally), *Uvaria rufa* (ornamental and medicinal), *Dillenia philippinensis* (endemic and ornamental), *Arisaema* sp. (ornamental), *Medinilla* sp. (rare and ornamental) and *Diospyros philippinensis* (endemic). Through the participation of the Subanons and Talaandigs, the abundance, local names and uses of the botanical resources were made possible. Some species considered to be endangered, endemic and economically important were saved through *in situ* and *ex situ* conservation. Findings of this study have led the community to identify the Nursery and Economic Garden as livelihood projects.

Keywords: flora, inventory, assessment, *ex situ* conservation

BSD No. 2

ALTITUDINAL GRADIENT DISTRIBUTION OF PTERIDOPHYTES ON MT. BANAHAW DE LUCBAN, LUZON ISLAND, PHILIPPINES

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An altitudinal transect study of pteridophytes was conducted at the northeastern slope of Mt. Banahaw de Lucban. Ninety-three (93) species representing 47 genera and 24 families were found in the study area. Fourteen percent (14%) of which are Philippine endemics. The most represented families are Polypodiaceae (11 spp.), Hymenophyllaceae (11 spp.) and Aspleniaceae (9 spp.) while the most represented genera are *Asplenium* (9 spp.), *Lycopodium* (5 spp.) and Selaginella (5 spp.). The pterido-flora of the mountain exhibits a strong Malesian floristic affinity.

Five altitudinal pteridophyte zones are proposed based on the results of cluster analysis and principal component analysis: **Zone 1,** *Cyathea contaminans – Dicranopteris - Nephrolepis - Diplazium* patches from 700 – 800 m a.s.l.; **Zone 2,** *Sphaerostephanos hirsutus* var. *hirsutus - Selaginella delicatula* patches from 750 – 900 m a.s.l.; **Zone 3,** *Cyathea philippinensis - Selaginella* patches from 900 – 1200 m a.s.l.; **Zone 4,** *Cyathea philippinensis - Cyathea*

callosa - Asplenium cymbifolium - Selaginella cumingiana patches from 1200 – 1550 m a.s.l. and; **Zone 5 which is further divided into Sub-zone 5A,** *Cyathea callosa - Cyathea loheri* - Hymenophyllaceae patches from 1550 – 1800 m a.s.l. and **Sub-zone 5B,** Cyathea loheri – Cephalomanes apiifolia patches from 1800 – 1875 m a.s.l. These pteridophyte zones coincide with the woody species zones and differ significantly with the altitudinal fern zones on Mt. Makiling.

Species diversity gradually increases with elevation, reaching a maximum at 814 – 886 m a.s.l. On the other hand, species cover did not show any direct relationship with altitude. Majority of the fern patches shelters all the pteridophyte height classes designated in this study. At least 85% of the pteridophyte species are preferential.

Stepwise multiple regression analysis reveals that altitude and soil pH exhibit a linear relationship with pteridophyte species distribution. Altitude and soil pH influences 65% of the variation in principal component 1 [PC1 = 0.0839 + 0.0010(altitude) - 0.2072(soil pH); r = 0.8058] and explains 27% of the variation in principal component 2 [PC2 = 2.0453 - 0.0005(altitude) - 0.2560(soil pH); r = 0.5206]. On the other hand, slope was found to be linearly related to species diversity, explaining 16% of the variation in H' [H' = 1.4928 + 0.0092(Slope); r = 0.3995]. The strong linear relationship expressed by pteridophyte distribution with elevation justifies the designation of altitudinal pteridophyte zones.

Keywords: Pteridophytes, distribution, Mt. Banahaw de Lucban

BSD No. 3

BIOLOGY AND CULTIVATION OF *Schizophyllum commune*, A NEWLY CULTIVATED PHILIPPINE EDIBLE MUSHROOM WITH NUTRICEUTICAL AND ANTIBACTERIAL PROPERTIES

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Our group studied the biology and cultivation of this wild edible mushroom *Schizophyllum commune* which may lead to the development of research - based production technology. Also, its nutriceutical and antibacterial properties were evaluated.

Spores of *S. commune* germinated best when immersed in rice bran decoction (pH 7.5) and incubated under illuminated condition at 32°C. Domestication studies showed that *S. commune* can be cultivated on natural substrates. Coconut water is the most appropriate culture medium for the mycelial production. Milled rice and palay seeds could support luxuriant mycelial growth and thus could serve as best mother spawning materials or starters. Its fruiting bodies grow best on a combination of sawdust and 5% rice bran. Among the logs evaluated, mango yielded quality fruiting bodies compared to ipil -ipil, rain tree and paper tree.

With regard to the nutriceutical and antibacterial properties, *S. commune* contains appreciable amount of protein (22%), crude fiber (3.59%), and carbohydrates (59.56 %) which merit it to be considered as nutritious food. The immobilized form of *S. commune* could overcome and suppress the growth and further colonization of *Staphylococcus aureus* and *Escherichia coli*.

These significant findings affirm that *S. commune* is a newly cultivated edible mushroom with nutriceutical and antibacterial properties.

Keywords: fungal flora, nutriceutical, Philippine fungi, *Schizophyllum commune*, wild edible mushroom

BSD No. 4

COMMUNITY STRUCTURE OF MACROPHYTES, BENTHIC MOLLUSCS, MEIOFAUNA AND MANGROVES IN THE EXPLOITED INTERTIDAL SAND FLAT IN DARUMAWANG IN PANGUIL BAY, MINDANAO

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The community structure of marine organisms was assessed in an exploited intertidal sandflat in Panguil bay. A stratified sampling method using transects lines and quadrats was employed to study the intertidal flat. Diversity indices, cluster analysis, detrended correspondence analysis, principal component analysis were used to evaluate the data collected in two sampling periods. Physicochemical parameters of the water and sediment of the area was also studied in relation to the distribution and abundance of organisms. Only one species of algae and one species of seaweed was found. There are two species of mollusks found in the area with 29 bivalves and 3 gastropods. The dominant species is *Modiolus metcalfei* followed by *Katylesia hiantina* and third is *Meretrix meretrix*. There are 11 groups of meiofauna present in the area

with nematoda as the dominant organism. Only two species of mangroves were found, Sonneratia and Avicennia. Multivariate analysis showed the community structure of the organisms in the intertidal flat generally has only few species as indicated by its low species richness value and are randomly distributed but not directly associated with the physicochemical properties of the sediments and water.

Keywords: community structure, Macrophytes, mollusks, seaweeds, mangrove, Panguil Bay

BSD No. 5

THE REPTILES OF MT. KIMANGKIL IN MINDANAO

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An inventory of reptiles in Mt. Kimangkil of Mindanao Island in the Philippines was conducted employing the cruising method. A total of 132 reptiles represented by 17 species, among which are Philippine endemics of 8 lizards and five snakes. Altitudinal distribution and habitat preferences of the collected species were noted while a preliminary data on their breeding season were collected. Overall assessment revealed that the presence of a large number of Philippine endemics in Mt. Kimangkil indicates that the area is of excellent quality providing habitat to a large number of reptiles including *Dasia griffini* and *Hologerrum philippinum* which are known to exist only in Palawan and Luzon respectively. The data presented are the first ever established for Mt. Kimangkil setting a milestone in the discovery of possible new subspecies in the area.

Keywords: reptiles, Mt. Kimangkil, *Dasia griffini*, *Hologerrum philippinum*

BSD No. 6

TAXONOMIC AND ECOLOGICAL STUDY OF PLANKTONS IN MARAGONDON RIVER, CAVITE

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A plankton study of Maragondon River, Cavite was conducted for classification and identification purposes. Three stations of 50 meters across the river were established. Plankton collection was done vertically and horizontally. Four parameters were observed: pH, temperature, dissolved oxygen, salinity, depth and current. Collected water samples were placed in a container preserved with 4% formalin. Plankton species present were analyzed using the Sedgewick Rafter counting chamber and a binocular microscope. The study obtained 14 genera of phytoplanktons and 9 genera of zooplanktons. *Coscinodiscus radiatus and Calanus helgolandicus* were the most abundant species of planktons. The Simpson index of diversity is 11.9 for phytoplanktons and 7.9 for zooplanktons indicating high diversity of planktons thriving in the river. pH , temperature and depth were recorded and correlated with the number of planktons counted.

Keywords: planktons, taxonomic, ecological, phytoplanktons, zooplanktons

BSD No. 7

GARGANTUAN LADYBIRD BEETLES OF THE PHILIPPINES (COLEOPTERA, COCCINELLIDAE, COCCINELLINAE, COCCINELLINI)

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Five species of ladybird beetles belonging to the tribe Coccinellini with a size ranging from 8mm to 13mm, *Anisolemnia reichi* (Mulsant), *Docimocaria commingi* (Mulsant, *Leis manillana* (Mulsant, *L. paulinae* (Mulsant) and *Synonycha grandis* Thunberg were described. These enormous ladybirds were found to be important predators of aphids. Four species were found to be endemic to the Philippines except for *S. grandis.*

Keywords: gargantuan, ladybird beetles, endemic, Coccinellini

BSD No. 8

FAUNAL INVENTORY IN THE MANGROVES AND MANGROVE COMMUNITIES OF THE THREE ISLANDS OF CAMOTES

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A faunal inventory in the mangrove areas of the Camotes group of Islands was conducted. There were eight mangrove areas where the study was conducted. Area 1 is Timarong, Poro, Cebu; Area 2 is Tiguis, Poro, Cebu; Area 3 is Unidos, San Francisco, Cebu: Area 4 is McArthur, Tudela, Cebu; Area 5 is Villahermosa, Tudela; Area 6 is Puertobello, Tudela, Cebu; Area 7 is Upper Poblacion, Pilar Cebu and Area 8 is Lower Poblacion, Pilar, Cebu. Sampling was done using a 600-m encircling net installed during high tide and harvested during low tide. Fish and invertebrates collected from each area were identified and individuals were counted. Result showed 74 species of fish inhabit the mangroves of Camotes belonging to 61 genera and 35 families. Twenty-five species of invertebrates were likewise found belonging to 19 genera and families. Among the areas sampled, area 2 was found to have the highest frequency of fish and invertebrates. The most dominant species of fish and invertebrates common to all areas are *Atherinomorous ogilbyi* and *Charbdis hawaiiensis*, respectively.

Keywords: mangroves, Camotes Islands, inventory, fauna.

BSD No. 9

SYNOPTIC REVISION OF SOUTHEAST ASIAN LAC INSECTS (KERRIIDAE, COCCOIDEA, HEMIPTERA)

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Eleven species of lac insects, including three previously known from the Philippines, are reported from Southeast Asia. *Tachardina aurantiaca* (Cockerell) is redescribed from Christmas Island, Indonesia and Malaysia and the Maldives. A species of *Kerria* from Vietnam is described as new to science. Diagnostic characters and distributional notes are provided for the rest of the species, namely: *Kerria (Chamberliniella) fici* (Green), *K.(C.) greeni* Chamberlin, *K.(C.) javana* Chamberlin, *K.(C.) rangoonensis* Chamberlin, *K.(K.) chinensis* (Mahdihassan), *K.(K.) lacca lacca* (Kerr), *K.(K.) lacca takahashii* Varshney, *Paratachardina minuta* (Morrison) and *Paratachardina* sp. Based on preliminary cladistic analyses, *Kerria* species from Southeast Asia form a monophyletic group, which is sister to the other *Kerria* species from India, Sri Lanka and the rest of Asia. A taxonomic key and illustrations are provided to facilitate their identification. Several reasons are offered and discussed as possible explanations for the seemingly depauperate lac insect fauna of this biogeographically significant region.

Keywords: lac insects, Hemiptera, Coccoidea, Kerriidae, *Kerria*, *Paratachardina*, *Tachardina*, taxonomy, biogeography

BSD No. 10

BIOLOGY AND POPULATION ABUNDANCE OF STRIPED FLEA BEETLE, *Phyllotreta striolata* Fab. (COLEOPTERA: CHRYSOMELIDAE) ON PAK-CHOI, *Brassica campestris* var. *chinensis*

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The striped flea beetle, *Phyllotreta. striolata* Fab. (Coleoptea:Chrysomelidae), is one of the major problem to pak-choi production in Central Luzon. Adult beetles fed on the cotyledon, surface leaves and produced

small pits, later these damage tissue breaks up producing a shot hole appearance. The severe infestation on emerging plants resulted to uneven growth and death of seedlings.

The biology of *P. striolata* was studied under laboratory condition at temperature and relative humidity ranged from 27 to 32°C and 52 to 38%, respectively. The population abundance was monitored on 8 crops of pak-choi from December 2002 to November 2003 at Experimental Research Area of Central Luzon State University, Science City of Muñoz, Nueva Ecija.

The total development period of *P. striolata* lasted for 18.35 ± 0.25 days, ranged from 16-18 days. The incubation period of the eggs lasted 3.53 ± 0.30 days (range, 3-5 days) with 73.1% hatchability. The *P. striolata* underwent three larval instars. The duration of the larval stadia are: 1st stadium, 2.54 ± 0.36 days (range, 2-4 days) second stadium, 2.83 ± 0.21 days (range, 3-5 days) and third stadium, 3.14 ± 0.14 days (range, 2-5 days). The prepupa and pupal stadia are: 2.30 ± 0.06 days (range, 2-3 days) and 4.02 ± 0.15 days (range, 3-6 days), respectively.

Adult longevity lasted for 39.68 ± 14.03 days (range, 21-62 days) in male and 36.56 ± 11.53 days (range, 20-59 days) in female. The mean female fecundity was 563.16 ± 149.24 (range, 325-961 eggs/female).

Chinese cabbage, *Brassica pekinensis* (Lour.) was the most preferred host plant, followed by pak-choi, *Brassica campestris var. chinensis* L. and Indian mustard, *Brassica juncea* L. The population of adult *P. striolata* was abundant in December to April with peak of population in March and declined in May. Population of *P. striolata* was not observed in June to August. Its population was significantly affected by rainfall. Results provided biological informations and population trends of *P. striolata*, which may contribute to the formulation of effective control measures in Central Luzon.

Keywords: *Phyllotreta striolata;* Development period; Population Abundance; Cruciferae; *Brassica campestris* var. *chinensis*

BSD No. 11

REPRODUCTIVE POTENTIAL OF COTTON BOLLWORM, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) AND ITS HOST ICHNEUMONID WASP, *Eriborus argenteopilosus* (HYMENOPTERA:ICHNEUMONIDAE)

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In the Philippines, cotton production is always threatened by the cotton bollworm *Helicoverpa armigera* Hubner (Lepidoptera:Noctuidae). This pest is considered as the most serious pests of cotton. It attacks the terminal buds, young leaves, flowers, squares and bolls causing a reduction in seedcotton yield by 28 to 97%.

In the Philippines, many naturally occurring parasitoids play an important role in reducing the population of the cotton bollworm, *Helicoverpa armigera* (Hubner) in cotton fields. They can be used to manage the pest in a sustainable way. One of the potential parasitod is the *Eriborus argenteopilosus*.

The reproductive potential of a parasitoid and its host is one of the factors to be considered in evaluating its performance as a biological control agent. A parasitiod is considered as a promising biological control agent when its reproductive potential is equal or higher than that of its host insect.

The study was conducted under screen house conditions for *H. armigera* and under laboratory conditions for *E. argenteopilosus*. The reproductive potential of the two insects were calculated using an age-specific life table analysis.

The intrinsic rate of increase (r_m) was 0.13 and the finite rate of increase in numbers (l) was 1.14 females/female/day with a net reproductive rate of 171.9.

A generation was completed in 39.7 days. Populations can increase weekly 2.5 times. When reaching the stable age distribution, *H. armigera* population age composition was 52.30% eggs, 18.94% first instar larvae, 12.38% second instar larvae, 5.53% third instar larvae, 4.57% fourth instar larvae, 2.10% fifth instar larvae, 1.42% sixth instar larvae, 2.31% pupae and 0.45% adults.

Eriborus argenteopilosus had a net reproductive rate of 36.2 and completed its generation in 21.4 days. The egg and larvae developed within 10.6 days and the pupa in 8.3 days. The intrinsic rate of increase and finite rate of increase in numbers were 0.17 and 1.18 females per female per day, respectively. The population of the wasp would be able to multiply 3.2 times every week.

The intrinsic rate of increase of *E. argenteopilosus* was 23 % higher than its host insect, *H. armigera*. Therefore, *E. argenteopilosus* can be an effective biological control agent for *H. armigera*.

Keywords: cotton, reproductive potential, *Helicoverpa armigera*, *Eriborus argenteopilosus*, intrinsic rate of increase, finite rate of increase

BSD No. 12

PHILIPPINE SPECIES OF *MESOCYCLOPS* (CRUSTACEA: COPEPODA) AS BIOLOGICAL CONTROL OF *AEDES AEGYPTI* (LINNAEUS)

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Rice fields, swamps, irrigation canals, and rivers in Luzon were surveyed for copepods. The following species were recovered: *Mesocyclops aspericornis* (Daday), *Mesocyclops ogunnus* Onabamiro (new record), *Microcyclops* sp., *Eucyclops* sp., *Thermocyclops decipiens* Keifer, and *Thermocyclops* sp.

Predatory capacity of local population of *Mesocyclops* species were evaluated, for the first time in the Philippines, as biological control of *Aedes*

aegypti (L.) mosquitoes. Under laboratory condition, *Mesocyclops* attacked the mosquito first instar larvae by the tail, side and head. Mean of first instar larvae consumed by *M. aspericornis* and *M. ogunnus* were 23.96 and 15.00 respectively. Analysis of variance showed that there was a highly significant difference between the mean number of first instar mosquito larvae consumed by *M. spericornis* and *M. ogunnus* which indicated that *M. aspericornis* is a more efficient predator of dengue mosquito larvae.

Larvitrap Index, Larval Density Index , and Larvitrap Density Index of Estero de Tanque showed that *Aedes aegypti* (65%) and *Aedes albopictus* (35%) were present in the area. House Index, Container Index and Breteau Index revealed that the area was sensitive for transmission of dengue. *Aedes* mosquitoes bred in indoor and outdoor containers such as plant vases, drums, used automobile tires, and plastic containers. KAP survey revealed that residents had insufficient information on dengue etiology, breeding sites, and biting habits of dengue mosquitoes.

Results of small scale field trials showed that the mean number of surviving larvae in experimental drums was 63.10 and 202.95 in control drums. T-test of means indicated that there was a significant difference between the mean number of surviving larvae in the drums with and without *M. aspericornis*. Findings indicated that *M. aspericornis* females are good biological control agents for they destroyed/consumed about two thirds of the wild, dengue mosquito larvae population.

Keywords: copepods, *Mesocyclops, Aedis aegypti,* dengue, mosqiotoes, biological control

BSD No. 13

ISOLATION AND CHARACTERIZATION OF *Vibrio spp.* FROM THE SEDIMENT OF CAGED AND UNCAGED SITES IN TAAL LAKE

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Fish kills have been frequently reported in several tilapia cage sites in Taal lake. Fermentative bacteria, particularly *Vibrio* spp. which include members potentially pathogenic to tilapia may be regarded as one of the causal suspects. This study reports on the isolation and characterization of *Vibrio*-like bacteria in the sediments of caged (Leviste) and uncaged (Quiling) sites in Taal Lake.

Four sediment samples from each site were pooled and from each two 5-gram replicate subsamples were taken for microbial analyses. The subsamples were diluted ten-fold in sterile saline solution. Aliquot (0.1 mL) of the dilutions were spread plated onto thiosulfate citrate bile salts sucrose (TCBS) agar plates and incubated at room temperature for 24 h. Colonies formed were regarded as putative vibrios. Discrete colonies were purified twice by streaking onto trypticase soy agar + 1.5 % NaCl (TSANa). The isolates were maintained on TSANa for characterization and identification. Morphological, physiological and biochemical tests were performed on the isolates. Twenty-four and forty-eight h cultures of the putative vibrios were tested for bioluminescent activity in TSANa and TCBS. Four *Vibrio*-like isolates were selected and further characterized using API20E and BIOLOG GN2 plate.

Twenty-six *Vibrio*-like bacteria were isolated from highly-diluted TCBS plate cultures. All the isolates were Gram-negative facultative anaerobic rods, straight and/or curved, and grow at temperatures 30 and 35 °C. All the isolates except one, catabolized glucose with acid production. Two isolates, both from the uncaged site, exhibited bioluminescent activity. This study reports for the first time the isolation of bioluminescent *Vibrio*-like bacteria from the sediment of Taal Lake.

The API20E and BIOLOG GN2 profiles of the bioluminescent and two other isolates from the caged site revealed resemblance of three isolates to non-*Vibrio* species, however, one bioluminescent isolate resembled *Vibrio vulnificus*, a pathogenic vibrio.

Keywords: Taal Lake, tilapia, *Vibrio*, bioluminescent bacteria

BSD No. 14

MORPHOLOGICAL CHANGES DURING THE STARVATION IN NILE TILAPIA *Oreochromis niloticus* L. LARVAE

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Starvation in considered an important cause of early mortality in fishes. In commonly grown food fish like nile tilapia, *Oreochromis niloticus* L, mortality is observed when newly-hatched larvae are starved for approximately 10 days. This study investigated the external morphology (length and weight) and internal morphology (height of cell layers in the intestine and stomach) of samples which were deprived of first feeding (exogenous feeding) for a maximum of eight days, had survived and were fed after starvation. One thousand newly-hatched larvae were equally distributed in down-welling basins and first fed at different times (fed upon hatching, fed after two days starvation, fed after four days starvation, fed after six days starvation, fed after eight days starvation). Feeding was administered in $1m^3$ fine-meshed "hapa" or net enclosure installed in fertilized earthen pond where the fish samples were reared. Sampling were done in an interval of two days for 30 days, where, after measurements of lengths and weights, gut samples were taken and processed in the laboratory using standard histological procedures. Nile tilapia fish larvae deprived of first feeding for a maximum of eight days had survived. However, growth and development of cells in the digestive tract which are determinants of efficient digestion were significantly delayed. Light microscopy had shown that the growth and developmental features of the digestive organs in older fish samples which were previously were comparable to those of the young samples which were fed immediately upon hatching, suggesting that delaying first feeding in nile tilapia larvae in fish farms should be avoided if quality of fish for consumption is to be considered an important factor in fish production.

Keywords: first feeding, fish larvae, *Oreochromis niloticus* L.

BSD No. 15

BIOREMEDIATION POTENTIAL OF CYANOBACTERIA AND MICROALGAE ISOLATED FROM SOME MINING AREAS IN THE PHILIPPINES

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Cyanobacteria and microalgae have been reported to survive in extreme environments. In the Philippines, metal pollution has become a growing concern due to effluents from mining sites and other industries. This study was conducted in order to determine the bioremediation potential of cyanobacteria and microalgae isolated from three mining sites, namely Baguio-Benguet Mining Corporation and Philex Mining Corporation in Benguet and Dizon Mines in Zambales. A total of 56 isolates were obtained. These isolates were classified as follows: 15 from Family Chroococales, 12 Oscillatoriales and 5f Nostocales. A rapid screening procedure for cadmium resistance was employed following the methodology of Matsunaga and co-workers (1999). This utilized microtiter plates inoculated with 2.5 mL of each strain in each well and incubated with varying $CdCl_2$ concentrations (0, 0.20, 2.0, 20, 50, 100, 500, 1000 Î¼M. Fifteen out of the 56 isolates were found to be able to survive up to a maximum concentration of 50 Î¼M $CdCl_2$. Further screening was done by incubating them in 50 Î¼M $CdCl_2$ for two weeks and determining the metal removal rate through Atomic Absorption Spectrophotometry. Isolate designated as Bnt 4a was computed to have the highest removal rate of 94.80%. Metal removal was correlated with the structural features of the organisms, where the cell wall can serve as the binding site of the cadmium cations.

Keywords: bioremediation, cyanobacteria, microalgae, cadmium resistance, atomic absorption spectrophotometry

BSD No. 16

MERCURY UPTAKE AND PHYTOCHELATIN PRODUCTION IN *IPOMOEA AQUATICA* FORSK.

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The Hg content of the vegetative organs and their subcellular fractions were determined in *Ipomoea aquatica* Forsk. plants grown in nutrient solutions supplemented with three levels of Hg, *viz.* : 0, 0.5, and 1.0 mg L^{-1}. The mercury-binding phytochelatin-like peptides involved in the uptake and accumulation of Hg were purified and quantified using reversed phase-high performance chromatography.

The increase in plant height and recovery in dry weight of *Ipomoea aquatica* plants grown in nutrient solutions with as high as 1.0 mg L^{-1} Hg for 7 days indicate a high degree of tolerance to Hg. The translocation of significant levels of Hg^{2+} that were 6- to 7-fold the levels found in the control plants or 65- to 75-fold the soil Hg, effectively took place in the young leaves. The levels of Hg^{2+} were higher in the total protoplasmic fractions than in the cell wall fractions. The presence of Hg^{2+} was detected in all subcellular fractions but higher levels were noted in the vacuoles and subsequently higher levels were noted in the vacuolar sap than in the tonoplast.

The sulfhydryl and glutathione-containing phytochelatin-like substances were detected mostly in the fractions from the young leaf extracts. The levels of phytochelatin-like peptides and the concentrations of Hg^{2+} have a direct relationship and are highest in the young leaves. The phytochelatin-like peptides were also detected at high levels in the stems and least in the mature leaves, although Hg^{2+} concentrations were higher in the mature leaves than in the stems.

These observations hint at oxidized glutathione and an accompanying derivative of the phytochelatin-like peptide as the chelating agent for the toxic heavy metal, mercury. The levels of oxidized GSH had been over-expressed in all vegetative organs, especially in the young leaves of the plants exposed to 1.0 mg L^{-1} Hg for 7 days.

Keywords: *Ipomoea aquatica*, mercury, phytochelatins, subcellular fractions, uptake

BSD No. 17

DETECTION OF PATHOGENIC AND NONPATHOGENIC STRAINS OF *Acanthamoeba spp.* THROUGH THE POLYMERASE CHAIN REACTION

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Acanthamoeba spp. is a group of opportunistic pathogens commonly found in the soil, and has been proven to cause several diseases in humans. DNA samples of environmental and clinical isolates of *Acanthamoeba* were extracted and amplified through PCR. To distinguish the pathogenic from the nonpathogenic strains of *Acanthamoeba*, the ac6 primer was used to generate PCR products indicative of the cyst's pathogenicity. Under less restrictive conditions, with an optimum annealing temperature of 56°C, bands of 200, 350, 400, 600, and 1000 bp were amplified from all the strains of *Acanthamoeba*. However, under restrictive conditions, with an optimum annealing temperature of 62°C, bands of 200 and 400 bp were produced only from the pathogenic Nh1 and Cot strains. This shows successful differential amplification of the ac6 locus based on the pathogenicity of the *Acanthamoeba* isolates. The banding patterns from the PCR products can also be used to establish genetic diversity based on the geographical location of the sources of the *Acanthamoeba* isolates.

Keywords: *Acanthamoeba*, PCR, pathogenicity

BSD No. 18

EFFECT OF HYDROGEN SULFIDE ON EXTRACELLULAR PROTEOLYTIC ACTIVITY IN MARINE SEDIMENTS

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Extracellular proteolytic activity (EPA) is a bottleneck in the recycling of nitrogen in marine sediments. H_2S presence, protein content, redox potential and EPA were determined in samples of sediment affected and unaffected by organic load from fish farming activities in Bolinao Bay, Pangasinan. Fish farming in net cages causes accumulation fish-feed borne proteins in marine sediments that have rapidly become oxygen-depleted. So to study the effect of H_2S and anoxic condition on EPA, we used an enzyme assay employing a dye-labeled scleroprotein as the enzyme substrate. The data suggest that redox potential was negatively correlated with protein content as well as with H_2S presence, whereas correlation analyses of H_2S presence vs. EPA showed a moderate negative correlation. Visual examination of some sulfidic samples showed confluent white mats of *Beggiatoa* indicating strong reooxidation of S^{2-} to elemental sulfur (S^o) in this type of sediment. EPA in the anoxic sediment with H_2S was significantly lower than the EPA of the oxic control but was nonsignificantly lower than the EPA in the anoxic sediment without H_2S incubation. Enzyme extract from cultured proteolytic bacteria incubated with increasing concentrations of H_2S, however, showed direct inhibition on EPA. The inhibitor constant obtained by method of Dixon plot was 20 mM. This indicates that though extracellular proteolytic enzymes could be directly inhibited by H_2S, they are less affected when contained in sediments. Furthermore, reooxidation of natural sediment originally containing H_2S would more likely decrease its EPA indicating that strictly anaerobic bacteria are governing this important microbial process in sulfidic sediments.

Keywords: H_2S, extracellular proteolytic activity, marine sediments, anoxia, sulfate reduction, nitrogen cycling, fish farming, mariculture, aquaculture, redox potential

BSD No. 19

EFFECTS OF ANTI-CD3 MONOCLONAL ANTIBODIES AND F(ab')$_2$ ON THE DOWN REGULATION AND INTERNALIZATION OF THE TCR/CD3 COMPLEX IN JURKAT CELLS

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Targeting of the T cell receptor/CD3 (TCR/CD3) complex on human T cells with anti-CD3 monoclonal antibodies (mAbs) has been used in preventing acute graft rejection for more than twenty years. Anti-CD3-mediated immunosuppression involves various levels of interference with T cell clonal activation that vary from physical blocking of antigen receptors, prevention of signal transduction, receptor internalization and/or downregulation, to the induction of apoptosis of preactivated T cells. Despite its potency, anti-CD3 therapies involving whole mAbs may be limited due to a number of factors such as initial Fc-mediated cytokine release, non-selective T cell killing, and immunogenic tolerance, which leads to non-responsiveness to the immunosuppressive therapy. Immunogenic tolerance is associated with the extensive internalization and/or downregulation of the TCR/CD3 complex resulting from the non-dissociation of high affinity anti-CD3 mAbs. In this study, we attempt to demonstrate the advantages of anti-CD3 F(ab')$_2$ over the whole mAb based on differing degrees of internalization and downregulation resulting from the administration of each. Western blot analysis was used to monitor the expression of CD3 in Jurkat cells over different periods of exposure to saturating and non-saturating concentrations of anti-CD3 mAb and F(ab')$_2$. Internalization will be monitored using flow cytometry. The removal of the Fc would make the F(ab')$_2$ dissociate from the TCR/CD3 more easily than the mAb *in vivo*, and it is expected that this would translate to less internalization and downregulation, thereby minimizing immunogenic tolerance.

Keywords: anti-CD3, monoclonal antibodies, F(ab')$_2$, graft rejection

BSD No. 20

GROWTH KINETICS OF *Oxytricha* sp. IN TWO CULTURE MEDIA AND ITS USE IN CYTOTOXICITY ASSAY

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Protozoans are ubiquitous organisms inhabiting aquatic, aerial, and terrestrial environments. Among those found in freshwater ponds is *Oxytricha* sp. which is a flexible, ellipsoid-bodied hypotrich that spends most of its time creeping on the substratum through its ventrally-located cirri. Clonal cultures of *Oxytricha* sp. were established and grown in two different media, namely, hay infusion and grass powder media where their growth kinetics were compared. It was found out that *Oxytricha* sp. exhibits better growth in grass powder medium than in the hay infusion medium with generation times of 13.2 hours and 21.1 hours, respectively. Range-finding toxicity test was also performed to determine the effects of a non-ionic surfactant, Triton X-100, on the behavior of this protozoan. The cells start to lyse when concentrations e" 50 ìg/mL were applied to the cultures. After a ten-minute exposure, no cells were found at the said concentrations. Moreover, the long-term toxicity effects of Triton X-100 were established through the cell count method and MTT assay. Through cell counting, LC_{50} of the said surfactant was found to be at 14.07 ìg/mL. However, based on the MTT assay, LC_{50} was 341.82 ìg/mL. Higher LC_{50} based on the MTT assay suggests the persistence of mitochondrial dehydrogenase activity even after cell lysis.

Keywords: *Oxytricha* sp., cytotoxicity, growth, protozoans

BSD No. 21

ANTIBODY AFFINITY AS A FUNCTION OF CHEMICAL REACTIVITY, STRUCTURAL PLASTICITY AND STABILITY

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Mutations introduced in antibody germline sequences as a result of somatic hypermutation could cause its derivatives to have an increased or decreased affinity for its target. Affinity maturation, however, favors the selection of the antibodies that exhibit increased affinity. In this study, we evaluate the effects of individual amino acid substitutions in relation to resultant chemical reactivities and affinities of selected sequences derived from a single germline. Physio-chemical properties, namely size, polarizability, polarity, charge, electrophilicity and electronegativity, are considered and taken in the context of its position in the antibody chain, as well as its exposure. Two-dimensional and three-dimensional modeling are also performed to further explain disparities in the affinities of sequences with very minimal differences in terms of replacement mutations. These, in turn, are correlated with the chemical properties of residues in the combining site, as well as the reorganizations that could be effected in this as a result of the presence of specific mutations.

Keywords: antibody affinity, mutation, chemical reactivity

BSD No. 22

CHITOSAN ACETATE INDUCES CELL MIGRATION AND TUBE FORMATION OF BOVINE AORTIC ENDOTHELIAL CELLS *IN VITRO*

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Initial studies have shown that chitosan, a structural biopolymer composed of repeating units of â-(1–4)deacylated glucosamine can induce a dose-dependent neovascularization in 8-day old chicken chorioallantoic membrane. Western blot analysis of the CAM lysate showed an increased expression of PDGFR, greater than the positive control bFGF. No detectable expression was observed for PDGFR, PECAM (CD31) and Flt-1. Migration assay with bovine aortic endothelial cells (BAEC) using Boyden Chamber confirmed that in the presence of chitosan acetate in DMEM where fibroblast (293) cells (lower chamber) were grown, more BAEC migrated into the outer surface of the upper chamber. Immunoassay of the 293 supernate revealed an increase drelease of IL-8, a chemoattractant cytokine and an angiogenic factor. This finding supports the dose dependent tube formation observed with BAEC when grown on Matrigel Basement Membrane Matrix with chitosan acetate.

Keywords: neovascularization, cell migration, tube formation , chemoattractant

BSD No. 23

CHEMOTHERAPEUTIC DRUG KILLS MACROPHAGES BY OVERSTIMULATION OF NO SYNTHASE

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Macrophages participate in the body's defense system by phagocytosis of microorganisms that may cause diseases. Superoxide radicals and NO production are generally increased during this activity. Certain chemotherapeutic drugs against cancer have been reported to kill immune response cells. In an attempt to determine if chemotherapeutic drugs against cancer kill macrophages by overstimulating free radical production, peritoneal murine macrophages were treated with different concentrations of the anticancer drug Taxol. This drug, causes mitotic arrest by affecting the assembly and disassembly of microtubules in spindle fibers. Thus they act on actively dividing cells. Peritoneal macrophages however, are not dividing cells. Thus inhibition of cell proliferation brought about by this drug must be caused by another pathway. In this study, when macrophages were incubated with Taxol or with Taxol together with lipopolysaccharide (LPS), a bacterial endotoxin established to stimulate the cells, a significant increase of NO production was observed. LPS alone serving as positive control, elicited only 1.98 μM of NO while LPS with increasing concentrations of Taxol elicited a range from 1.9 to 9.8 μM of NO. Addition of N^G-Monomethyl L-arginine (L-NMMA), known to inhibit the activity of NO synthase, was observed to significantly lower the production of NO. With L-NMMA, the amount of NO produced ranged from 0.5 to 2.5 μM only. Direct cell viability count was done to determine if there is a correlation between cell viability and NO production. Cells treated with 100 μg/ml Taxol and LPS or with 100 μg/ml Taxol alone, showed 50% viability. Treatment with L-NMMA rescued cells from death with viability at the same drug concentration going up to 70%. This suggests that the chemotherapeutic drug enhances NO production which at extremely high level may cause death of the macrophages.

Keywords: macrophages, superoxide radicals, NO synthase, lipopolysaccharide, L-NMMA

BSD No. 24

CHEMOPROTECTIVE EFFECTS OF *Amaranthus gracilis* AND *Beta vulgaris* ON SELECTED ORGANS OF TUMOR-INDUCED MICE

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To find possible cures for cancer, researches have been directed to the study of the possible uses of animal and plant extracts. This study was aimed at determining any protective effects of *Amaranthus gracilis* (spinach) and *Beta vulgaris* (beets) extracts on some organs, namely the skin, spleen, kidneys, and liver, of tumor-induced mice. Swiss Webster albino mice were treated previously with DBMA, croton oil, and spinach and beet extracts using three different protocols (one hour prior to croton oil application and five days before DMBA application, one hour prior to croton oil application only, and immediately after croton oil application). The mice that survived were further subjected to paraffin processing and histopathological analysis. The organs treated with spinach and beet extracts showed histodegeneration like the positive control but in varying degrees, depending on the protocol used. The kidneys, liver, and skin were protected by the extracts. The least damage was seen in the organs treated with extracts one hour prior to croton oil application and five days before DBMA application.

Keywords: *Amaranthus gracilis, Beta vulgaris*

BSD No. 25

IMMUNOMODULATORY ACTIVITY OF MICE INJECTED WITH HYDROSOLUBLE EXTRACT OF *Chlorella pyrenoidosa*

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Chlorella pyrenoidosa is a unicellular green algae that is commonly researched for its therapeutic properties and is known to perform an immonumodulatory activity. An indicator of the presence of an immunomodulatory activity is phagocytosis. This experimental study was designed to test for the toxic properties of *Chlorella* and its ability to increase the phagocytic activity of macrophages. Also, it aimed to determine if the cytotoxicity of *Chlorella* and its effect on phagocytosis are dependent on exposure time to *Chlorella*. A hydrosoluble extract of *Chlorella pyrenoidosa* was injected intraperitoneally to mice. The control group was injected with distilled water. Mice were given different exposure times (one day, four days, eight days, and twelve days) to *Chlorella* and distilled water. The mice were sacrificed after their assigned exposure time. Macrophages were collected from the peritoneal cavity of the mice and were subjected to tests. To assess the cytotoxicity of the *Chlorella* extract, trypan blue exclusion test was done. Cytotoxicity was observed based on the percent viability of the macrophages. Regardless of exposure time, the values for percent viability obtained from the mice injected with *Chlorella* and distilled water were of the same range - 84.27% to 88.176%. Therefore, *Chlorella* showed no indication of cytotoxicity in all of the exposure times observed. Yeast assay was done to test for *Chlorella*'s effect on phagocytosis. *Chlorella*'s effect on phagocytosis was dependent on exposure time since *Chlorella* increased the phagocytic activity of the macrophages only on the exposure time of four days. On this exposure time, mice injected with *Chlorella* had an average percent phagocytosis of 61.81%. Those injected with distilled water had an average percent phagocytosis of 37.98%. The other exposure times showed no significant difference between the percent phagocytosis of mice injected with *Chlorella* and those injected with distilled water.

Keywords: *Chlorella pyrenoidosa*, hydrosoluble, phagocytosis, cytotoxicity

BSD No. 26

TUMOR PROGRESSION AND IMMUNOLOCALIZATION OF MONOCLONAL ANTIBODY CC49 IN A BALB/C MOUSE MODEL

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Tumor-associated glycoprotein 72 (TAG-72) is an antigen widely expressed in human carcinomas. CC49 is a G1 allotype (IgG1) monoclonal antibody (Mab) highly reactive with TAG-72. In this study, the immunotherapeutic potential of MAb CC49 was assessed in a Balb/C mouse model. 4T1 murine mammary carcinoma cells expressing TAG-72 were injected subcutaneously into Balb/C mice to induce the formation of primary tumors, which were then allowed to metastasize to the different organs of the mice. In order to determine the immunolocalization and efficacy of CC49 in reducing the growth of primary tumors, biotinylted CC49 was injected intraperitoneally into the mice. Tumor progression was monitored by weighing the mice and measuring the volumes of primary tumors from the injection of 4T1 cells up to three weeks after MAb administration. From the data gathered in the tumor progression study, there is no evident reduction in tumor growth by CC49. immunohistochemical analysis is still being performed on the primary tumors and sample organs to determine the localization and binding of the biotinylated CC49.

Keywords: tumor progression, immunolocalization, TAG-72, CC49 carcinoma

BSD No. 27

AGE-ASSOCIATED CHANGES IN THE QUANTITY OF m_1 MUSCARINIC ACETYLCHOLINE RECEPTORS (mAChR) IN THE HIPPOCAMPUS OF THE RAT BRAIN

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Muscarinic acetylcholine receptors (mAChRs) mediate cholinergic transmission in the cortex and hippocampus and play a role in higher brain processes such as learning, memory, arousal and motor control. A heterogenous family of five genetically distinct mAChR subtypes is present in the brain (m_1, m_2, m_3, m_4 and m_5). All five mAChR genes are expressed in the hippocampus, and subtype-specific antibodies have enabled identification, quantification, and localization of the encoded proteins. In the hippocampus and several regions of neocortex in human brain, m_1 ranges from 35-60% of all mAChR binding sites. The objective of this study is to compare the quantity of m_1 mAChR subtype in the CA_1 region of rat hippocampus of different ages (young, mature and old). The presence of m_1 mAChR subtype in the hippocampus of the rat brain was investigated using SIGMA anti-muscarinic acetylcholine receptor (M_1) M-9808. Frozen sections of the hippocampus region at 4, 7 and 10 micrometer were obtained using a cryostat which were treated with m_1 receptor subtype antibody at concentrations of 1:1500 and 1:3000.

Results show positive labeling of m_1 mAChR subtype in the hippocampus which are dispersed in the different regions of the hippocampus proper. Comparing the relative quantity of m_1 mAChR subtype in the CA_1 region of the hippocampus, the result follows a normal curve where the brain of the mature rat manifests the greatest quantity of m_1 mAChR subtype. m_1 mAChR subtype is found to be more abundant in the entorhinal cortex and subiculum region than in the hippocampus proper and this may be due to the distribution of cholinergic cell groups and the pathway of acetylcholine in the brain. The characterization of receptor subtypes and their localization would be relevant in defining targets for the development of more effective and specific therapeutic drugs for neurological diseases.

Keywords: m_1 mAChR subtype, rat, rat brain, hippocampus, immunohistochemistry

BSD No. 28

ANGIOGENIC PROPERTY OF *Aloe barbadensis* MILLER (ALOE VERA) LEAVES

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The sap from the leaves of Aloe vera (*Aloe barbadensis Miller*) is commonly used in wound treatment and induction of hair growth. To scientifically document these applications of Aloe vera, the present study aims to determine its potential angiogenic property using the chorioallantoic membrane assay (CAM). The sap of aloe vera leaves was extracted with different solvents such as water, methanol and ethyl acetate. Chicken eggs incubated for nine days were treated with dose dependent concentrations of the extracts. At the twelfth day of incubation CAM was harvested and viewed under the microscope at low power objective (4x). The ethyl acetate extract exhibited a potential stimulatory effect on angiogenesis, it showed an increase in blood vessel branching suggesting that it contains anigiogenic stimulant compounds. The dialyzed water extract showed that the inhibition of neovacularization decreased, implying the role of ions as angiogenic inhibitor. The methanol extract exhibited reduced number of branching on angiogenesis in the CAM assay indicating that this extract may contain compounds that inhibit angiogenesis. The angiogenic property of Aloe vera can be regarded either as an inducer which can be found in the ethyl acetate extract and as an inhibitor which can be found in water and methanol extracts.

Keywords: *angiogenesis, Aloe barbadensis Miller, chorioallantoic membrane assay*

BSD No. 29

LOCAL PLANT CRUDE EXTRACTS WITH INHIBITORY ACTIVITY AGAINST EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL)-PRODUCING *E. coli* AND *K. pneumoniae* AND OXACILLIN-RESISTANT (ORSA) *S. aureus*

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The use of herbal plants for medicinal treatment has been the most common option in the country, owing most to the wild-growing plants for their curing abilities. This study was conducted exploring the activities of our locally abundant plants such as the *Codiaeum variegatum, Hibiscus rosasinensis, Anona muricata and Imperata cylindrica.* The ability of each of these plants to inhibit the growth of some clinically significant bacteria such as *E.coli, K. pneumoniae* and *S. aureus* was done by single disk diffusion technique, using first the normally susceptible strains of these organisms. The plant extracts that were found to be highly active were further assayed against the confirmed multi-drug resistant extended-spectrum beta-lactamase (ESBL)-producing *E.coli* and *K. pneumoniae,* and further with the oxacillin-resistant *S. aureus* (previously known as MRSA). Also, potential synergy between these highly active plant extracts was determined by double-disk synergy technique (DDST), to be able to come up with new approach in the use of herbal plants, which may then help prevent possible development of resistance among these pathogenic strains.

Analysis of the bioconstituents of these plants by thin-layer chromatography revealed the presence of alkaloids, saponins, tannins, anthroquinones, higher alcohols, steroids and essential oils. Disk-diffusion assay of the plant extracts showed that all have inhibitory activity against *E. coli, K. pneumoniae* and *S. aureus.* Significantly, extracts of *Hibiscus rosasinensis, Anona muricata and Imperata cylindrical* were observed to be equally potent against ESBL-producing *E.coli* and *K. pneumoniae* and oxacillin-resistant *S. aureus,* with *Hibiscus rosasinensis noted to be the most active showing zones of inhibition ranging from 25 to 35 mm.* Further, possible synergy between plants was seen in double-disk synergy technique. No antagonistic activity has been noted.

Keywords: medicinal plants, *Codiaeum variegatum, Hibiscus rosasinensis, Anona muricata, Imperata cylindrica*

BSD No. 30

IDENTIFICATION AND LOCALIZATION OF THREE mAChRs SUBTYPES IN RAT HIPPOCAMPUS USING IMMUNOHISTOCHEMISTRY

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The muscarinic acetylcholinergic receptors (mAChRs) and its subtypes belong to a large class of integral membrane glycoproteins that mediate cholinergic processes like learning, memory and attention. In this study, three antibodies against mAChR subtypes (m1, m3, and m4 subtypes) were used to identify and localize mAChRs in the rat hippocampus. Separate frozen sections of the hippocampal region at 4, 7 and 10 microns were obtained using a cryostat and were treated with the antibodies at two concentrations (1:1500 and 1:3000) using immunohistochemistry. Results show that only m1 mAChR subtype showed positive labeling in the hippocampus region with the presence of brown-stained cells while m3 and m4 mAChR subtypes both showed negative labeling with the presence of blue-stained cells. This indicates that m3 and m4 mAChR subtypes might not be synthesized nor transported in the hippocampus unlike the m1 mAChR subtype.

Keywords: rat, rat brain, hippocampus, mAChRs, immunohistochemistry

BSD No. 31

MARINE NATURAL PRODUCTS THAT INDUCE APOPTOSIS IN THE MCF-7 BREAST CANCER CELL LINE

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The potential of marine resources in biomedical research has been recognized through the utilization of marine organisms as sources of novel compounds with significant pharmacologic activity. A number of novel natural

products have been purified from marine sponges and marine microorganisms. Cytotoxic studies of these compounds in the human breast cancer cell line MCF-7 revealed significant anticancer activity. To determine whether apoptosis is the mechanism behind the observed cytotoxic activities, cellular morphology, DNA fragmentation, and cell cycle analysis were done through Hoechst staining, DNA laddering assay, and flow cytometry, respectively. Results showed characteristic morphology and DNA fragmentation expected of apoptotic cells. Flow cytometry also showed an increase in the percentage of apoptotic cells. These support the hypothesis that apoptosis is the most likely mechanism behind the cytotoxic activities observed in some of these compounds.

Keywords: apoptosis, MCF-7, marine sponges, cytotoxic activities

BSD No. 32

CHARACTERIZATION OF TUMOR-ASSOCIATED GLYCOPROTEIN, TAG-72, FROM HUMAN COLORECTAL CANCER TISSUES

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Tumor-associated antigens sudh as (TAG-72) are studied, most particularly, because of their potential role in cancer immunodiagnosis and as target for various immunotherapeutic strategies. TAG-72 is a useful surface tumor marker and diagnostic molecule because it is expressed in a variety of malignant epithelial tumors but not in normal tissues. In order to develop a TAG-72-based cancer diagnostic kit, the isolation of the TAG-72, of optimal purity, is required. With a number of paraffinized block samples from two patients, the CC49 monoclonal antibody (Mab) reactive TAG-72 was detected employing immunohistochemical (IHC) staining diaminobenzidine (DAB) solution. Employing a heat extraction step, lysates containing TAG-72 in Tris-buffered saline (TBS) were prepared. Partial purification using a G75-Sephadex gel filtration column and preliminary detection through sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were done. The homogenates were further purified in a CNBr-activated CC92-Sepharose 4B affinity column. Western blotting showed positive lysate immunoactivity, signifying the presence of the antigenic

determinant TAG-72. This was further verified in an enzyme-linked immunosorbent assay, wherein a linear relationship of the log antigen concentration against absorbence at 410nm was obtained. Separation in SDS-PAGE is done prior to in-gel trypsin digestion of the high molecular weight band. On a separate aliquot of the purified samples, deglycosylation using periodate prior to trypsin digestion is done. Both digests will be analyzed by mass spectrometry necessary for possible peptide sequencing.

Key words: tumor-associated glycoprotein, TAG-72, colorectal cancer

BSD No. 33

ISOLATION OF THEONELLAPEPTOLIDE ID FROM A BATANES SPONGE *Theonella* sp.

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The crude extract of a *Theonella* sp. sponge collected from Batanes, Philippines showed significant antitumor activity against human breast cancer cell lines MCF7 and SKBR3 and also anti-TB activity. This study aimed to isolate, purify and elucidate the compound(s) responsible for such activity. The sponge was extracted with methanol and subjected to a modified Kupchan solvent partitioning. The anti-TB activity and tumor cytotoxicity were concentrated in the chloroform fraction. The bioassay-guided isolation of the active compound from the chloroform fraction was performed by successive Sephadex LH-20 and C18 flash column chromatography and solvent partitioning. The off-white crystal showed an MIC of 1 μg/mL in MABA and an IC_{50} of 6.49μg/mL in the MCF7 breast cancer cell line. The MS-ESI spectrum revealed a cluster around m/z 1421.7 and the melting point was determined to be 128°C. IR analysis showed the presence of carbonyl and amino groups distinctive of peptides. From the MS-MALDI spectrum the isolate was shown to contain two major peptides and four minor compounds. This was further purified by HPLC using a Phenomenex C18

column and a water:methanol gradient system. The major HPLC fraction was subjected to ^{1}H-NMR and ^{13}C-NMR spectroscopy and the molecular weight determined by MS-MALDI spectrometry. Spectroscopic data were comparable with those reported for Theonellapeptolide Id. The pure compound showed cytotoxicity in MCF7 and SKBR3 breast cancer cell lines at 5μg/mL with a fractional survival of 0.298 and 0.072, respectively.

Keywords: *Teonella*, antitumor, anti-TB, sponge

BSD No. 34

THE MANOBO, HIGAUNON AND THE BADJAO LUMAD MALES: HOW DIFFERENT ARE THEY PHYSICALLY?

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Variability among select male indigenous peoples specifically the Manobo of Pangantucan, Higaunons of Kalabugao and the sea dwellers, the Badjaos were assessed based on sixteen phenotypic traits, five (5) anthropometric measurements and seventeen (17) dermatoglyphic variables. Some distinct phenotypic traits were uniquely present in the groups. Short little finger was found present in the Higaunons and not in individuals from other tribes. The Manobos from Pangatucan did not have alleles for dimples; however half of the Higaunons in Kalabugao had dimples. Many recessive traits were observed expressed in frequency values higher than those of the dominant traits. This result was consistent with ethnohistorical and cultural practices of the indigenous peoples, of which they preferred endogamous marriages. Differences in anthropometric measurements were observed. Higaunons were found to have the greatest mean values of all anthropometric measurements. Differences between dermatoglyphic characters such as fingerprint traces, palmar loopings, triradius distribution, and certain complicated ridge-patterns were also observed. Unique loopings and whorls were also observed at the hypothenar region and Palm Area 1 of certain individuals. Homogeneity and heterogeneity of individuals in some variables provide better insights into the population structure and history of subdivision, which are consistent with the known ethnohistorical backgrounds of the populations.

Keywords: dermatoglyphic characters, anthropometric, Higaunon, Monobo, Badjao

BSD No. 35

UNDERSTANDING DIFFERENCES BETWEEN MEN AND WOMEN: BODY MORPHOMETRICS, ASYMMETRY AND ATTRACTIVENESS

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For centuries, differences between men and women are socially defined. While there are obvious differences in the two sexes in gross morphology, very few studies have dwelled on specific body morphometric attributes, asymmetry and attractiveness. We investigated variations between sexes based on anthropometric measurements of the hand, foot, face, mendelian traits and fingerprints from 600 subjects (300 males and 300 females). Results showed that symmetry was higher for males than females for foot length, hand length, index finger, middle finger, little finger, ear length and position. Asymmetry from hand width and thumb length was higher in females than in males. There was a relationship between asymmetry and trait size in most characters evaluated. Based on face morphometrics, ear width, lip width and thickness, nose length, length from base of nose to tip, back of head to eye point, back of head to sidemost of the eye, length from top of head to eye, nose base to tip, frontmost of ear to eye point, neck base to tip of chin, and frontmost of ear to sidemost of the eye are significantly different between males and females. For Mendelian traits, men have higher frequency of dominant traits while females have higher frequencies of recessive traits. Alcoholism was prevalent among males while anemia was observed to be predominant among females. Many disorders like cleft palate, club foor, harelip and those suffering from leukemia were predominant in males while heart disease, hypertension, manic depression, schizophrenia and tuberculosis were predominant in females. This study also showed that attractiveness of either sex is not primarily associated with other body indices but on face features. Both sexes preferred asymmetric faces than symmetric ones although computer reconstruction of the original image of the face showed that symmetric faces are better looking than the original asymmetric face.

Keywords: body morphometrics, asymmetry, male, female

BSD No. 36

CALCIUM CRYSTALS IN LEAVES OF SOME AMARANTHACEAE OF THE PHILIPPINES

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The family Amaranthaceae is a widely distributed family of herbs, shrubs or small trees comprising 65 genera and over 1,000 species. In the Philippines, there are about 12 genera and about 21 species which are widely distributed from Luzon to Mindanao. Some species are used as vegetables, as ornamentals, as medicinals, as common weeds and as excellent fodder-plants. Species have slightly astringent properties, others are diaphoretics and diuretics, though many have use in native practice as alteratives and as antidotes to snakebite. Crystals in plants may be a storage form of calcium when the availability of calcium increases in the soil. It is also suggested as defense mechanism against predators and could also be species specific. This study involved an investigation of the presence of crystals in leaves of 19 species and two varieties belonging to six genera of the family Amaranthaceae. Light microscopy studies was done using the BH-2 Olympus epifluorescent microscope and the CK10 Olympus inverted microscope equipped with camera. Histological techniques used were the modified clearing and paraffin techniques. Cleared and cross-sections of leaves showed the presence of three types of crystals- the flower-like druse crystals, geometric prismatic and sand crystals in 6 species namely: *Amaranthus caudatus, A. spinosus, A. tricolor, A. virides, Alternanthera amoena,* and *A. frutescens.* Ten species showed two types of crystals, the druse and prismatic in *Amaranthus gangeticus, Gomphrena celosiodes, G. glogosa, Alternanthera dentata, A. repens, A. versicolor, A. sp, Celosia argentea, Cyathula prostata, and Iresine herbstii.* Five species showed only one type - the prismatic crystals for the following species: *Celosia argentea "Castle Gould", C. cristata, C. cristata "purple", C. cristata "white" var.* and *Gomphrena sp.* Since the Amaranthaceae are considered economically important plants with its varying use as medicinal, nutritional, food and ornamentals, renewed interest in plant crystals specifically in the leaves is essential as it may serve as a tool or guide in recommending plants for medicine, and calcium as dietary supplement.

Keywords: crystals, calcium oxalate, druse, prismatic, sand crystals, Amaranthaceae

BSD No. 37

GAMMA-IRRADIATED CARRAGEENAN AS A GROWTH PROMOTING AGENT IN *Pleurotus florida* (ANGEL MUSHROOM)

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Carrageenan, a leading Philippine export product, is a family of linear and highly sulfated marine polysaccharides. Recent radiation research on carrageenan suggest that its radiolytic products (or partially degraded species) can serve as potent growth-promoting agents on a number of plants, like *Oryza sativa*. We report that this growth phenomenon can be demonstrated also on *Pleurotus sajor caju* and *Pleurotus florida*, two popular favorite mushroom varieties, both *in vitro* and *in vivo*. This enhanced productivity from our pilot-scale mushroom production studies may be due to the accelerated rate of mycelial colonization of the substrate containing trace amounts of 100-kGy Irradiated kappa- and iota-carrageenan.

These promising results provide a new strategy for the mushroom industry to profitably promote its production by optimal use of radiation-modified substrates.

Keywords: Kappa-carrageenan, Iota-carrageenan, *Pleurotus florida*, Growth promoter

BSD No. 38

DIRECT ACCLIMATIZATION OF *IN VITRO* CULTURED *GRAMMATOPHYLLUM SCRIPTUM* (ORCHIDACEAE)

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Shortening the *in vitro* culture period and speeding the acclimatization process of a Philippine native orchid, *Grammatophylum scriptum* were explored. Response of *G. scriptum* protocorms ex *vitro* in closed systems to the difference of time of opening was specifically studied. *G. scriptum* protocorms directly planted on fern slabs (5cm x 5cm) were placed inside a sealed beverage plastic bottle (1.5 L) for 12 weeks. Set-ups were placed at random on compot beds under natural light. Caps were consequently opened at two weeks interval for 1 1/2 months and measurements were made on the 8th week thereafter. Protocorms grew and developed into green and sturdy plantlets. Shoot length, number of protocorms with shoots, number of protocorms, number of leaves per shoot and percent survival increased with time. The difference of values of these parameters except for the percent survival between 8th, 10th, and 12th weeks were computed to be not significant. This is attributed to the small opening made by the cap, which probably did not contribute a notable effect with a closed system. Hence, the results show that it is feasible for *G. scriptum* protocorms to grow and develop *ex vitro*. This will address the problem of the long *in vitro* residence. In addition, this promotes early hardening and minimizes acclimatization mortality.

Keywords: *Grammatophylum scriptum*, Philippine orchid, protocorms, direct acclimatization, *in vitro* residence, culture period, *ex vitro*, growth and development

BSD No. 39

EMBRYOGENESIS AND ORGANOGENESIS OF *PHALAENOPSIS SP.* (ORCHIDACEAE)

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This study determined the concentration of plant hormones and kind of explant for the organogenesis and embryogenesis of *Phalaenopsis.* Different explants were cultured for sixteen (16) weeks (2 weeks-dark:14 weeks-light) on modified Knudson Formula C medium supplemented with different levels of NAA, BAP and combination of both. Callus and organ formations were achieved at 1-2 ppm NAA, 1-4 ppm BAP and of both. Shoot explants showed better response than leaf tip, root tip, root base and leaf base. On the other hand, some calli have developed from leaf tip failed to differentiate while others had leaf formation. No significant differences were found in the length of shoots among all treatments. 52% of shoot explants turned green while the rest were vitrified. This experiment also demonstrated that *Phalaenopsis* shoot explants could differentiate into full-developed shoots even without the presence of plant growth regulators. This may be attributed to the presence of meristematic tissues in shoots and the addition of hormones merely enhances organogenesis.

Keywords: *Phalaenopsis sp.* explants, Orchidaceae, embryogenesis, organogenesis, *in vitro* culture, modified Knudson Formula C medium

BSD No. 40

DIRECTIONAL CLONING STRATEGY FOR THE CONSTRUCTION OF A PLANT GENE EXPRESSION VECTOR

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A full length chitinase gene was cloned from a Philippine strain *Serratia marcescens* considered as a bacterial entomopathogen. Chitinase is one of the main hydrolytic enzymes used by *Serratia* spp. during insect pathogenesis. For the prokaryotic chitinase gene to be expressed in eukaryotic systems such as

plants, a suitable plant expression cassette is very important. The expression cassette is based on a co-transformation system where the gene-of-interest (GOI) and the antibiotic selectable marker gene are physically separated. The chitinase gene from a TOPO cloning vector was transferred to a pGTVa expression vector using a directional cloning approach. Initially, the chitinase gene was re-amplified and cloned into the TOPO cloning vector. Specific restriction enzymes were used to cut specifically the chitinase gene and the pGTVa for directional ligation. The ligation product was transformed in *E. coli* and the plasmid minipreps verified for the presence of the chitinase gene. COLONY-PCR, molecular weight size and COLDSTART-PCR were used to verify the presence of chitinase gene. A directional strategy will fast-track the construction of a plant gene expression cassette and we are now ready to transfer the chitinase gene into corn in our effort to develop a transgenic corn with insect resistance to the Asiatic corn borer.

Keywords: directional cloning, chitinase, *Serratia marcescens*, expression cassette, corn borer

BSD No. 41

ONTOLOGY AND ISOFORM DISCOVERY OF GENES INVOLVED\ IN FATTY ACID SYNTHESIS IN COCONUT (*Cocos nucifera* L.)

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Coconut (*Cocos nucifera* L.) is the major export crop of the Philippines due to its vegetable oil which is rich in medium-chain fatty acids. Several genes are involved in fatty acid synthesis in coconut. Among them are: acyl-ACP thioesterase (TE), phosphatidic acid phosphatase (PAP), acyl carrier protein (ACP), acetyl CoA carboxylase (ACCase), lysophosphatidic acid acyl transferase (LPAAT) and ß-keto acyl (ACP) synthase 3 (KAS 3). To isolate and detect for the presence of isoforms of each gene at the 4,5 and 6 mo old coconut endosperms and to establish an ontological significance of the genes involved in fatty acid synthesis, the 3'RACE method was used.

For TE, two bands were detected in the 5 and 6 mo. old coconut endosperms. For PAP, three bands were detected in the 6 mo old coconut endosperm. For ACP, a single band was detected in the 4 mo old coconut endosperm. For ACCase, 2 bands from the 4 mo. old and 8 bands from the 6 mo old coconut endosperms. For LPAAT, 2 bands were detected in the 6 mo old coconut endosperm. For KAS 3, 2 bands were detected from the 5 and 6 mo old coconut endosperms.

The results obtained indicate the presence of each of the genes and their isoforms at varying ages of the coconut endosperm. Furthermore, the results obtained show an ontological pattern of significance in the expression of genes involved in fatty acid synthesis in coconut.

Keywords: coconut, medium chain fatty acid , isoform, RACE (Randomly Amplified cDNA Ends), fatty acid synthesis

BSD No. 42

MOLECULAR ANALYSIS OF RESVERATROL SYNTHASE GENE IN PEANUT (*Arachis hypogaea* L.)

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The transformation of resveratrol synthase (RS) into important crops has been an attractive option since it is the key enzyme in the synthesis of resveratrol (3, 4', 5-trihydroxystilbene), a stilbene phyoalexin that has anti-leukemic, antioxidant and chemopreventive properties. Therefore, the gene encoding the enzyme for resveratrol biosynthesis is a very important gene not only in agriculture but also in the field of health and medicine. The full length RS gene was isolated and cloned using genomic DNA from germinating seeds of peanut (*Arachis hypogaea* L.) by PCR. A 1.5 kb PCR product was generated using RS-specific primers. Multiple sequence alignment of the isolated genes showed that they have high similarity with each other and with known RS genes. Further analysis revealed the presence of two exons (exon 1: 180 bp and partial exon 2: 197 and 670 bp) and one intron (331 bp). The conserved MVSVSG and

RSMAI that flanked the RS gene were also evident based on the DNA sequence of RS. Approximately 150 bp of sequence data was missing due to limitations of PCR cycle sequencing and this missing region contain the highly conserved active site (cys_{166}) predicted to be involved in the condensing reaction of a polyketide synthase. Further sequencing using an internal primer should be done to obtain the full sequence of the isolated RS genes. Non-random differences in the nucleotide sequence alignment and in the partial restriction sites in both exons and introns were observed suggesting the presence of at least two RS genes in peanut. In conclusion, a full length and functional resveratrol synthase gene was cloned from peanut and we are now in the position to transfer this gene to important crops by genetic engineering. Stable expression of the RS gene in plants may lead to enhanced protection from microbial infections and increasing their nutraceutical value.

Keywords: molecular analysis, resveratrol synthase, peanut, nutraceutical value

BSD No. 43

PARTIAL CHARACTERIZATION AND MOLECULAR CLONING OF SWEETPOTATO FEATHERY MOTTLE VIRUS (SPFMV)

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Sweetpotato feathery mottle virus (SPFMV) has been identified as one of the most important constraints in sweetpotato production. The virus has been noted in large sweetpotato fields in Central Luzon. Spreading over most of the sweetpotato areas, it has led to substantial yield losses and the loss of an important variety called "Bureau".

Several SPFMV isolates have been characterized based on differential reactions to diagnostic hosts, *Ipomoea setosa* and *I. nil.* The virus was purified from mechanically inoculated *I. nil* using cesium chloride (Cscl) step gradient centrifugation giving a faint opalescent band near the bottom of the centrifuge tube. The virus yield ranged from 9-30 mg /kg with A 260nm/A280nm ratio of

around 1.2. The purified virus was infectious and exhibited flexuous rod particles typical of a potyvirus under an electron microscope.

Further characterization of the SPFMV isolates by reverse transcriptase polymerase chain reaction (RT PCR) of the purified virus resulted in the amplification of the coat protein gene of SPFMV using two sets of primers designed to amplify the partial and full length coat protein gene of SPFMV. The expected PCR product sizes of 400/bp and 1.0 kb for partial and full length CP, respectively were obtained and successfully cloned using the TOPO TA cloning kit of INVITROGEN.

Such results on the characterization and cloning of SPFMV would be very useful in illuminating its position within the potyvirus group. Moreover, information on the molecular aspects of the virus would help facilitate the development of rapid and sensitive techniques for virus detection and identification which are important in monitoring virus infection in the field.

Keywords: sweetpotato feathery mottle virus (SPFMV), potyvirus, cloning, purification, primers, coat protein gene (CP), reverse transcriptase polymerase chain reaction (RT PCR), sweetpotato.

BSD No. 44

MOLECULAR MAPPING OF GRAIN QUALITY QUANTITATIVE TRAIT LOCI IN RICE (*Oryza sativa* L.) BY SELECTIVE GENOTYPING USING SIMPLE SEQUENCE REPEATS

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Two advanced backcross inbred lines of rice (*Oryza sativa* L.), RF 52 (IR 64 x Karnal Local) and RF 57 (Tequing x Basmati) were characterized based on ten grain quality traits. Quantitative trait loci (QTL) controlling grain quality were also determined by selective genotyping using Simple Sequence Repeats. Sixty-nine putative QTLs controlling grain quality traits were detected, 42 for RF52 and 27 for RF 57. These QTLs are widely distributed over the 12 chromosomes of rice and together explains the phenotypic variation for the analyzed grain

quality traits in the two populations. QTLs for amylose content were located in chromosomes 3 and 5 (RF 52) and 8 (RF 57). Three QTLs each for gel consistency were identified in chromosomes 2 and 11 for the two crosses and the rest were identified in chromosomes 3,4,6 (RF 52) and 5 (RF 57). QTLs for gelatinization temperature were located in chromosomes 1,2,4 (RF52), 2 and 8 (RF 57); for aroma in 1,7,8 (RF 52), 9,10 and 11 (RF57); for cooked kernel length in 1,4,5,10 (RF 52), 2,5 7 and 12 (RF 57); for cooked kernel width in 5,6,7,8,10 11 (RF52) and 10 (RF 57); for cooked kernel length-width ratio in 5,6,7,8,10,11 (RF 52) and two in 7 and one in 12 (RF 57); for uncooked kernel length in 1,6,9,11,12 (RF 52), 2,5 and 10 (RF 57); for uncooked kernel width in 3,4,8,10,11 (RF52), 2,6,8 and 11 (RF 57); and for uncooked length-width ratio in 6,9 (RF 52) 2 and 6 (RF 57).

Keywords: quantitative trait loci, simple sequence repeats, grain quality, amylose, cooked kernel length and width

BSD No. 45

DETERMINATION OF GENETIC VARIATION IN GINGER (ZINGIBERACEAE) THROUGH RAPD ANALYSIS OF THE CHLOROPLAST DNA (cpDNA)

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Chloroplast DNA (cpDNA) is oftenly used in the field of plant systematics. In this study, Ribulosebisphosphate carboxylase large sub-unit (rbcL) primers are used in the analysis of genetic differentiation of different ginger (Zingiberaceae) species through random amplification of polymorphic DNA (RAPD) of the isolated cpDNA. The genomic DNA from sixteen ginger species found in the University of the Philippines Diliman campus was initially extracted. Using polymerase chain reaction (PCR), rbcL primers were applied to amplify the rbcL gene in the cpDNA of each sample. Another cycle of PCR was then performed using RAPD primers to assess genetic diversity among the ginger species. Using cluster analysis, species having similar banding patterns are more closely related compared to those with varying patterns. A dendogram was also produced to aid in the assessment of the relationship among the ginger species.

Keywords: Zingiberaceae, ginger, phylogeny, ribulosebisphosphate carboxylase large sub-unit (rbcL), random amplification of polymorphic DNA (RAPD), chloroplast DNA (cpDNA)